भारतीय मानक
Indian Standard

IS 16147 : 2014
(Reaffirmed 2020)

कैराजीनन, खाद्य ग्रेड — विशिष्टि

Carrageenan, Food Grade — Specification

ICS 67.220.20

© BIS 2014

भारतीय मानक ब्यूरो
BUREAU OF INDIAN STANDARDS
मानक भवन, 9 बहादुरशाह ज़फर मार्ग, नई दिल्ली–110002
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI-110002
www.bis.org.in www.standardsbis.in

February 2014 **Price Group 5**

Food Additives Sectional Committee, FAD 08

FOREWORD

This Indian Standard was adopted by the Bureau of Indian Standards, after the draft finalized by the Food Additives Sectional Committee had been approved by the Food and Agriculture Division Council.

Carrageenan (C.A.S. number 9000-07-1) [*Synonyms* — Irish moss gelose (from *Chondrus* spp.); Eucheuman (from *Eucheuma* spp.); Iridophycan (from *Iridaea* spp.); Hypnean (from *Hypnea* spp.); Furcellaran or Danish agar (from *Furcellaria fastigiata*); INS No. 407] is a substance with hydrocolloid properties obtained from certain members of the class *Rhodophyceae* (red seaweeds). The principal commercial sources of carrageenans are the following families and genera of the class of *Rhodophyceae*:

Furcellariacaea such as *Furcellaria*
Gigartinaceae such as *Chondrus*, *Gigartina*, *Iridaea*
Hypnaeceae such as *Hypnea*
Phyllophoraceae such as *Phyllophora*, *Gynmogongrus*, *Ahnfeltia*
Solieriaceae such as *Eucheuma*, *Anatheca*, *Meristotheca*.

Carrageenan is a hydrocolloid consisting mainly of the ammonium, calcium, magnesium, potassium and sodium sulphate esters of galactose and 3,6-anhydrogalactose polysaccharides. These hexoses are alternately linked α-1,3 and β-1,4 in the copolymer. The relative proportions of cations existing in carrageenan may be changed during processing to the extent that one may become predominant.

The prevalent polysaccharides in carrageenan are designated as kappa-, iota-, and lambda-carrageenan. Kappa-carrageenan is mostly the alternating polymer of D-galactose-4-sulphate and 3,6-anhydro-D-galactose; iota-carrageenan is similar, except that the 3,6-anhydrogalactose is sulphated at carbon 2. Between kappa-carrageenan and iota-carrageenan there is a continuum of intermediate compositions differing in degree of sulphation at carbon 2. In lambda-carrageenan, the alternating monomeric units are mostly D-galactose-2-sulphate (1,3-linked) and D-galactose-2,6- disulphate (1,4-linked).

Carrageenan is obtained by extraction from seaweed into water or aqueous dilute alkali. Carrageenan may be recovered by alcohol precipitation, by drum drying, or by precipitation in aqueous potassium chloride and subsequent freezing. The alcohols used during recovery and purification are restricted to methanol, ethanol and isopropanol.

Carrageenan is used as a thickener, gelling agent, stabilizer and emulsifier. Articles of commerce may include sugars for standardization purposes, salts to obtain specific gelling or thickening characteristics, or emulsifiers carried over from drum drying processes.

This standard, which is based on JECFA standard for Carrageenan, prepared at the 68th JECFA (2007) and published in FAO JECFA Monographs 4 (2007), has been formulated to provide the necessary guidelines to manufacturers and the consumers for maintaining quality control.

In the preparation of this standard, due consideration has been given to the *Legal Metrology (Packaged Commodities) Rules*, 2011 and *Food Safety and Standards Act,* 2006 and Rules framed thereunder. The standard is however subject to restrictions imposed under these rules wherever applicable.

For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the results of a test or analysis, shall be rounded off in accordance with IS 2 : 1960 'Rules for rounding off numerical values (*revised*)'. The number of significant places retained in the rounded off value should be the same as that of the specified values in this standard.

Indian Standard

CARRAGEENAN, FOOD GRADE — SPECIFICATION

1 SCOPE

This standard prescribes requirements, methods of sampling and test for carrageenan, food grade.

2 REFERENCES

The following standards contain provisions, which through reference in this text, constitute provisions of this standard. At the time of publication, the editions indicated were valid. All standards are subject to revision and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below.

IS No.	*Title*
1070 : 1992	Reagent grade water (*third revision*)
1699 : 1995	Methods of sampling and test for synthetic food colours
2491 : 2013	Food hygiene — General principles — Code of practice (*third revision*)
5402 : 2012	Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of micro-organisms — Colony-count technique at 30°C
5887	Methods for detection of bacteria responsible for food poisoning:
(Part 1) : 1999	Isolation, identification and enumeration of *Escherichia coli*
(Part 3) : 1999	General guidance on methods for the detection of *Salmonella*

3 REQUIREMENTS

3.1 Description

Yellowish or tan to white, coarse to fine powder that is practically odourless.

3.2 Identification Tests

3.2.1 *Solubility*

The material shall be insoluble in ethanol and shall be soluble in water at a temperature of about 80°C, forming a viscous clear or slightly opalescent solution that flows readily when tested according to method prescribed in Annex A. The material shall disperse in water more readily if first moistened with alcohol, glycerol, or a saturated solution of glucose or sucrose in water.

3.2.2 *Sulphates*

Dissolve a 100 mg sample in 20 ml of water (with heating if necessary), and add 3 ml of barium chloride test solution (TS*) and 5 ml of hydrochloric acid, dilute test solution (TS#); filter if a precipitate forms. Boil the solution or the filtrate for 5 min. A white, crystalline precipitate shall appear.

3.2.3 *Galactose and Anhydrogalactose*

Galactose and anhydrogalactose shall be present when tested according to Annex B.

3.2.4 *Identification of Hydrocolloid and Predominant Type of Copolymer*

Add 4 g of sample to 200 ml of water, and heat the mixture in a water bath at 80°C, with constant stirring, until dissolved. Replace any water lost by evaporation, and allow the solution to cool to room temperature. It becomes viscous and may form a gel. To 50 ml of the solution or gel add 200 mg of potassium chloride, then reheat, mix well, and cool. A short-textured ('brittle') gel indicates a carrageenan of a predominantly kappa type, and a compliant ('elastic') gel indicates a predominantly iota type. If the solution does not gel, the carrageenan is of a predominantly lambda type.

3.2.5 *Infrared Absorption*

The material shall have strong, broad absorption bands, typical of all polysaccharides, in the 1 000 to 1 100 cm^{-1} region when tested according to Annex C. Absorption maxima shall be 1 065 and 1 020 cm^{-1} for gelling and non-gelling types, respectively. Other characteristic absorption bands and their intensities relative to the absorbance at 1 050 cm^{-1} shall be as given in Table 1.

3.3 The material shall also conform to the requirements given in Table 2.

3.4 The material shall be processed, packed, stored and distributed under hygienic conditions in licensed premises (*see* IS 2491)

---

* [12 percent (*w/v*) solution of barium chloride ($BaCl_2 \cdot 2H_2O$) in water (approximately N)]

[#] Solution containing 10 percent *w/v* of HC1, prepared by diluting 266 ml of hydrochloric acid (36 percent) with sufficient water to make 1 000 ml.

Table 1 Characteristics of Absorption Band

(*Clause* 3.2.5)

Wave Number (cm^{-1})	Molecular Assignment	Absorbance Relative to 1 050 (cm^{-1})		
		Kappa	Iota	Lambda
(1)	(2)	(3)	(4)	(5)
1220-1260	ester sulphate	0.3-1.4	1.2-1.7	1.4-2.0
928-933	3,6-anhydrogalactose	0.2-0.7	0.2-0.4	0-0.2
840-850	galactose-4-sulphate	0.2-0.5	0.2-0.4	—
825-830	galactose-2-sulphate	—	—	0.2-0.4
810-820	galactose-6-sulphate	—	—	0.1-0.3
800-805	3,6-anhydrogalactose-2-sulphate	0-0.2	0.2-0.4	—

Table 2 Requirements for Carrageenan

(*Clause* 3.3)

Sl No.	Characteristic	Requirement	Method of Test, Ref to	
			Annex of this Standard	Indian Standards
(1)	(2)	(3)	(4)	(5)
i)	Loss on drying, percent by mass, *Max*	12	D	—
ii)	*p*H	8-11	E	—
iii)	Viscosity (at 75°C of 1.5 percent solution), centipoise, *Min*	5	F	—
iv)	Sulphate (as SO_4^{2-}), percent by mass	15-40	G	—
v)	Total ash (on dry basis), percent by mass	15-40	H	—
vi)	Acid insoluble ash (on dry basis), percent by mass, *Max*	1	J	—
vii)	Acid insoluble matter (on dry basis), percent by mass, *Max*	2	K	—
viii)	Residual solvents, percent by mass of ethanol, isopropanol, or methanol, singly or in combination, percent by mass, *Max*	1	L	—
ix)	Arsenic (as As), mg/kg, *Max*	3.0	—	**15** of IS 1699
x)	Lead (as Pb), mg/kg, *Max*	5	—	**15** of IS 1699
xi)	Cadmium (as Cd), mg/kg, *Max*	1.5	—	**15** of IS 1699
xii)	Mercury (as Hg), mg/kg, *Max*	1	—	**15** of IS 1699
xiii)	Total (aerobic) plate count, cfu/g, *Max*	5 000	—	IS 5402
xiv)	*Salmonella* spp.	Absent	—	IS 5887 (Part 3)
xv)	*E. coli*	Absent (in 1g)	—	IS 5887 (Part 1)

4 PACKING, STORAGE AND MARKING

4.1 Packing

The material shall be securely packed in well-filled containers with minimum access to air. The containers shall be such as to preclude contamination of the contents with metals or other impurities.

4.2 Storage

The material shall be stored in a cool and dry place so as to avoid excessive exposure to heat.

4.3 Marking

Each container shall be legibly and indelibly marked with the following information:

a) Name of the material including the words 'Food Grade';
b) Name and address of the manufacturer;
c) Batch or Code No.;
d) Net quantity when packed;
e) Instruction for storage;
f) Best before date (month and year to be given by the manufacturer); and
g) Any other requirements as specified under the *Legal Metrology (Packaged Commodities) Rules,* 2011 and *Food Safety and Standards (Packaging and Labelling) Regulation*, 2011 and the Rules framed thereunder.

4.3.1 *BIS Certification Marking*

The product may also be marked with the Standard Mark.

4.3.2 The use of the Standard Mark is governed by the provisions of the *Bureau of Indian Standards Act*, 1986 and the Rules and Regulations made thereunder. The details of conditions under which the licence for the use of the Standard Mark may be granted to manufacturers or producers may be obtained from the Bureau of Indian Standards.

5 SAMPLING

Representative samples of the material shall be drawn according to the method prescribed in **4** of IS 1699.

6 QUALITY OF REAGENTS

6.1 Unless specified otherwise, pure chemicals and distilled water (*see* IS 1070) shall be employed in tests.

NOTE — 'Pure chemicals' shall mean chemicals that do not contain impurities, which affect the experimental results.

ANNEX A

(*Clause* 3.2.1)

TEST OF SOLUBILITY

A-1 PROCEDURE

Transfer a known amount of the sample into a flask containing known amount of the specified solvent, shake for no less than 30 s and no more than 5 min.

A-2 INTERPRETATION

Approximate solubilities are to be interpreted according to Table 3.

Table 3 Interpretation of Solubility

(*Clause* A-2)

Descriptive Term (1)	Parts of Solvent Required for 1 Part of Solute (2)
Very soluble	Less than 1
Freely soluble	From 1 to Less than 10
Soluble	From 10 to Less than 30
Sparingly soluble	From 30 to Less than 100
Slightly soluble	From 100 to Less than 1 000
Very slightly soluble	From 1 000 to Less than 10 000
Practically insoluble or in soluble	More than 10 000

ANNEX B

(*Clause* 3.2.3)

TEST FOR GALACTOSE AND ANHYDROGALACTOSE

B-1 PROCEDURE

Boil a mixture of 200 mg of the sample and 20 ml of 10 percent sulphuric acid for 3 h. Allow to cool and add excess barium carbonate, mixing with a magnetic stirrer until the solution is *p*H 7, and filter. Evaporate the filtrate in a rotatory evaporator at 30-50°C under vacuum until a crystalline (or syrupy) residue is obtained. Dissolve in 10 ml of 40 percent methanol. This is the hydrolysate. Place 1 to 5 µl spots of hydrolysate on the starting line of two Silica Gel G thin layer plates. On the same plates apply 1 to 10 µg of the reference standards of galactose, rhamnose, galacturonic acid, 3, 6-anhydrogalactose, mannose, arabinose and xylose.

Develop one plate in solvent A and one plate in solvent B:

a) A mixture of formic acid, methyl ethyl ketone, tertiary butanol and water (15/30/40/15 by volume); and
b) A mixture of glacial acetic acid/chloroform/water (74/65/11 by volume).

After development spray with a solution of 1.23 g anisidine and 1.66 g phthalic acid in 100 ml ethanol and heat the plates at 100°C for 10 min. A greenish yellow colour is produced with hexoses, a red colour with pentoses and a brown colour with uronic acids. Compare sample spots with those for the solutions of the reference standards and identify the constituents.

ANNEX C

(*Clause* 3.2.5)

TEST FOR INFRARED ABSORPTION

C-1 PROCEDURE

Disperse 2 g of the sample in 200 ml of 2.5 percent potassium chloride solution, and stir for 1 h. Let stand overnight, stir again for 1 h, and transfer into a centrifuge tube. (If the transfer cannot be made because the dispersion is too viscous, dilute with up to 200 ml of the potassium chloride solution.) Centrifuge for 15 min at approximately 1 000 (rpm) g.

Remove the clear supernatant, resuspend the residue in 200 ml of 2.5 percent potassium chloride solution, and centrifuge again. Coagulate the combined supernatants by adding 2 volumes of 85 percent ethanol or isopropanol (Retain the sediment for use as directed below). Recover the coagulum, and wash it with 250 ml of the alcohol. Press the excess liquid from the coagulum, and dry it at 60ºC for 2 h. The product obtained is the non-gelling fraction (lambda-carrageenan).

Disperse the sediment (retained above) in 250 ml of cold water, heat at 90°C for 10 min, and cool to 60ºC. Coagulate the mixture, and then recover, wash, and dry the coagulum as described above. The product obtained is the gelling fraction (kappa- and iota-carrageenan).

Prepare a 0.2 percent aqueous solution of each fraction, cast films 0.5 mm thick (when dry) on a suitable non-sticking surface such as Teflon, and obtain the infrared absorption spectrum of each film. (Alternatively, the spectra may be obtained using films cast on potassium bromide plates, if care is taken to avoid moisture).

ANNEX D

[*Table* 2, *Sl No.* (i)]

TEST FOR LOSS ON DRYING

D-1 PROCEDURE

Mix the sample well and accurately weigh 1 g of the substance. Reduce the sample to a fine powder. Tare a glass-stoppered, shallow weighing bottle that has been dried for 30 min at 105ºC to constant weight. Transfer the sample into the bottle, replace the cover, and weigh the bottle and the sample. Distribute the sample as evenly as practicable to a depth of about 5 mm. Place the bottle with its contents in the drying chamber, removing the stopper and leaving it also in the chamber and dry the sample at 105ºC to constant weight. Upon opening the chamber, close the bottle promptly and allow it to come to room temperature in a desiccator before weighing.

NOTE — Suitable precautionary steps should be taken when weighing hygroscopic or deliquescent samples to ensure that they do not absorb moisture.

ANNEX E

[*Table* 2, *Sl No.* (ii)]

DETERMINATION OF *p*H (POTENTIOMETRIC METHOD)

E-0 PRINCIPLE

The *p*H of an aqueous solution may be determined accurately by potentiometry using a *p*H meter. The practical definition of *p*H in water may be given by the equation:

$$pH = pH^o + [(E - E^o) / 0.059\ 1]$$

where

pH = value for the solution being measured;

pH^o = value for a standard buffer;

E = potential value for the solution being measured;

E^o = potential value for the standard buffer; and

0.059 1 = value at 25° of the Nernstian constant.

The equation does not apply to solvents other than water, or to mixed solvents that include water. However, the *p*H meter gives reproducible readings in other solvent systems, on the basis of calibration with aqueous buffers, and while the *p*H readings lack thermodynamic significance they are useful in setting specifications.

The measurement of *p*H using a *p*H meter is a matter of comparing the meter reading of an unknown solution with the meter readings of standard buffers whose *p*H values are accurately known. Standard buffer solutions are described in compendia, such as the Merck Index. Routine measurement uses only one buffer and an approximation of the electrode slope, usually made by a temperature compensator, *p*H measurement accurate to ± 0.05 *p*H unit or better requires the use of two buffers that bracket, if possible, the expected *p*H range. All samples and buffer should be at the same temperature.

The choice and care of glass and reference electrodes must be carefully considered. The ordinary glass electrode begins to be sensitive to alkali metal cations at *p*H values above about 9, leading to the so-called alkaline error. Electrodes with a greatly reduced alkaline error should be used for readings in the alkaline range. Store the electrodes in distilled water when not in use, in order to avoid dehydration. 'Flow-type' electrodes may be used if evidence of validity of *p*H measurement with the electrode is demonstrated.

The measurement of the *p*H of 'highly buffered solutions' (distilled water or solutions of non-ionic organic compounds in distilled water) is a particularly difficult measurement. The addition of 0.3 ml of a saturated solution of potassium chloride per 100 ml of distilled water helps by providing a small amount of electrolyte. However, it will usually be necessary to protect the solution being measured from the carbon dioxide in air by use of a blanket of nitrogen during the measurement. Measure the *p*H of successive portions of the distilled water or test solutions, with vigorous agitation, until the observed results for two successive portions agree within 0.1 *p*H unit.

E-1 PROCEDURE

Use a suitable *p*H meter and follow the manufacturer's instructions. Each time the electrodes are used, rinse them with distilled or deionised water and carefully blot them dry with clean absorbent tissue. Form a fresh reference electrode liquid junction. Rinse the sample vessel three times with each new solution to be introduced.

Choose two standard buffers (standard buffer solutions are described in compendia, such as the Merck Index) to bracket, if possible, the anticipated *p*H of the unknown. Warm or cool these standards as necessary to match within 2° the temperature of the unknown, and initially set the temperature compensator to that temperature. Immerse the electrodes in a portion of the first standard buffer, and following the manufacturer's instructions adjust the appropriate standardization control (knob, switch, or button) until the *p*H reading is that of the buffer. Repeat this procedure with fresh portions of the first standard buffer until two successive readings are within ± 0.02 *p*H unit without an adjustment of the standardization control.

Rinse the electrodes, blot dry, and immerse them in a portion of the second standard buffer of lower *p*H. Do not change the setting of the standardization control. Following the manufacturer's instructions, adjust the slope control (thumbwheel switch, knob, or temperature compensator) until the exact buffer *p*H is displayed.

Repeat the sequence of standardization with both buffers until the *p*H readings are within ± 0.02 *p*H unit for both buffers without any adjustment of either control (the amount of sample to be used in sample preparation is given where applicable in the individual specification.). The *p*H of the unknown solution may then be measured (The difference between the results of two *p*H determinations when carried out simultaneously on in rapid succession by the same analyst, under the same conditions, should not exceed 0.05 *p*H unit.)

Always re-standardize the instrument after even a short period during which the amplifier is turned off.

ANNEX F

[*Table* 2, *Sl No.* (iii)]

DETERMINATION OF VISCOSITY

F-1 PROCEDURE

Disperse an accurately weighed 15 g sample of commercial product into 500 ml of 60 percent (*w/w*) isopropanol/water at room temperature. Stir gently for 4 h. Filter through ash-free filter paper. Discard the filtrate. Wash the material remaining on the filter paper with two 15 ml portions of 60 percent isopropanol/water. Dry the material at 105°C to constant weight. Transfer 7.5 g of the dried sample obtained into a tared, 600 ml tall-form (Berzelius) beaker, and disperse with agitation for 10 to 20 min in 450 ml of deionized water. Add sufficient water to bring the final weight to 500 g, and heat in a water bath with continuous agitation, until a temperature of 80°C is reached (20-30 min). Add water to adjust for loss by evaporation, cool to 76-77°C, and heat in a constant temperature bath at 75°C.

Pre-heat the bob and guard of a viscometer to approximately 75°C in water. Dry the bob and guard, and attach them to the viscometer, which should be equipped with a No. 1 spindle (19 mm in diameter, approximately 65 mm in length) and capable of rotating at 30 rpm. Adjust the height of the bob in the sample solution, start the viscometer rotating at 30 rpm and, after six complete revolutions of the viscometer, take the viscometer reading on the 0-100 scale.

If the viscosity is very low, increased precision may be obtained by using the Brookfield UL (ultra low) adapter or equivalent. Record the results in centipoises, obtained by multiplying the reading on the scale by the factor given by the Brookfield manufacturer.

NOTE — Samples of some types of carrageenan may be too viscous to read when a No. 1 spindle is used. Such samples obviously pass the specification, but if a viscosity reading is desired for other reasons, use a No. 2 spindle and take the reading on the 0-100 scale or on the 0-500 scale.

ANNEX G

[*Table* 2, *Sl No.* (iv)]

DETERMINATION OF SULPHATE

G-0 PRINCIPLE

Hydrolyzed sulphate groups are precipitated as barium sulphate.

G-1 PROCEDURE

Disperse an accurately weighed 15 g sample of commercial product into 500 ml of 60 percent (*w/w*) isopropanol/water at room temperature. Stir gently for 4 h. Filter through ash-free filter paper. Discard the filtrate. Wash the material remaining on the filter paper with two 15 ml portions of 60 percent isopropanol/water. Dry the material at 105°C to constant weight.

Accurately weigh 1 g of the dried matter. Transfer the sample to a 100 ml long-neck round-bottom flask. Add 50 ml of 0.2 N hydrochloric acid. Fit a condenser, preferably one with at least 5 condensing bulbs, to the flask and reflux for 1 h. Add 25 ml of a 10 percent (by volume) hydrogen peroxide solution and resume refluxing for about 5 h or until the solution becomes completely clear. Transfer the solution to a 600 ml beaker, bring to a boil, and add drop wise 10 ml of a 10 percent barium chloride solution. Heat the reaction mixture for 2 h on a boiling water bath. Filter the mixture through ash free slow filtration filter paper. Wash with boiling distilled water until the filtrate is free from chloride. Dry the filter paper and contents in a drying oven. Gently burn and ash the paper at 800°C in a tared porcelain or silica crucible until the ash is white. Cool in a desiccator. Weigh the crucible containing the ash.

G-2 CALCULATION

Calculate the percentage sulphate from the weight in g (W_2) of the ash (barium sulphate) using the formula:

$$\frac{W_2 \times 100 \times 0.4116}{W_1}$$

where

W_1 = weight of material taken for test, in g; and

W_2 = weight of the ash, in g.

ANNEX H

[*Table* 2, *Sl No.* (v)]

DETERMINATION OF TOTAL ASH

H-1 PROCEDURE

Disperse an accurately weighed 15 g sample of commercial product into 500 ml of 60 percent (*w/w*) isopropanol/water at room temperature. Stir gently for 4 h. Filter through ash-free filter paper. Discard the filtrate. Wash the material remaining on the filter paper with two 15-ml portions of 60 percent isopropanol/water. Dry the material at 105°C to constant weight. Accurately weigh 2 g of the dried sample (W_1). Transfer to a previously ignited, tared silica or platinum crucible. Heat the sample with a suitable infrared lamp, increasing the intensity gradually, until the sample is completely charred; continue heating for an additional 30 min. Transfer the crucible with the charred sample into a muffle furnace and ignite at about 550ºC for 1 h. Cool in a desiccator and weigh. Repeat the ignition in the muffle furnace until a constant weight (W_2) is obtained. If a carbon-free ash is not obtained after the first ignition, moisten the charred spot with a 1-in-10 solution of ammonium nitrate and dry under an infrared lamp. Repeat the ignition step.

H-2 CALCULATION

$$\text{Calculate the percentage of total ash of the sample} = \frac{W_2 \times 100}{W_1}$$

where

W_1 = mass of the crucible with the material before drying, in g; and

W_2 = mass of the crucible with the material after drying and after it has come to room temperature in g.

ANNEX J

[*Table* 2, *Sl No.* (vi)]

DETERMINATION OF ACID INSOLUBLE ASH

J-1 PROCEDURE

Boil the ash obtained as directed under determination of total ash (*see* **H-1**), with 25 ml of dilute hydrochloric acid for 5 min, collect the insoluble matter on a suitable ash-less filter, wash with hot water, ignite at 800 ± 25°C, cool and weigh. Calculate the percentage of acid-insoluble ash from the weight of the sample taken.

ANNEX K

[*Table* 2, *Sl No.* (vii)]

DETERMINATION OF ACID INSOLUBLE MATTER

K-1 PROCEDURE

Disperse an accurately weighed 15 g sample of commercial product into 500 ml of 60 percent (*w/w*) isopropanol/water at room temperature. Stir gently for 4 h. Filter through ash-free filter paper. Discard the filtrate. Wash the material remaining on the filter paper with two 15 ml portions of 60 percent isopropanol/water. Dry the material at 105°C to constant weight.

Transfer 2 g of the dried sample, accurately weighed, into a 250 ml beaker containing 150 ml of water and 1.5 ml of sulphuric acid test solution. Cover the beaker with a watch glass and heat the mixture on a steam bath for 6 h rubbing down the wall of the beaker frequently with a rubber-tipped stirring rod and replacing any water lost by evaporation. Weigh 500 mg of a suitable acid washed filter aid, pre-dried at 105°C for 1 h, to the nearest 0.1 mg, add this to the sample solution and filter through a tared Gooch crucible provided with an asbestos pad. Wash the residue several times with hot water, dry the crucible and its contents at 105°C for 3 h, cool in a desiccator and weigh. The difference between the total weight and the weight of the filter aid plus crucible and pad is the weight of the acid-insoluble matter. Calculate as percentage.

ANNEX L

[*Table* 2, *Sl No.* (viii)]

DETERMINATION OF RESIDUAL SOLVENTS BY HEAD SPACE CHROMATOGRAPHY

L-0 PRINCIPLE

Headspace-gas chromatography (headspace-GC) is an analytical technique in which the analyte in its gas phase and its GC analysis have been combined. The method can be applied to the analysis of compounds with a low boiling point, which therefore vapourize easily at low temperatures.

There are two different forms of headspace-GC: static and dynamic headspace-GC. In static headspace-GC the gaseous sample is taken from a sealed headspace vial into a loop and the analytes are transferred to the GC with the help of carrier gas. In dynamic headspace-GC the gaseous sample is forced out of the headspace vial with the help of an external source, usually the same gas as that used as the carrier gas. Headspace injectors are equipped with a thermostatically controlled sample-heating chamber. Solid or liquid samples in tightly closed headspace vials incubated in the chamber for a fixed period of time, allowing the volatile components in the sample to reach equilibrium between the non-gaseous phase and the gaseous or headspace phase. After incubation at a specified temperature a gas sample from the head-space vial is sampled.

Purge and trap injectors are equipped with a sparging device by which volatile compounds in solution are carried into a low-temperature trap. When sparging is complete, trapped compounds are thermally desorbed into the carrier gas by rapid heating of the temperature-programmable trap.

L-1 REAGENTS

L-1.1 Blank — Sample with very low solvent content

L-1.2 Internal Standard — 3-methyl-2-pentanone

L-1.3 Methanol

L-1.4 Demineralized Water

L-1.5 Internal Standard Solution

Add 50.0 ml water to a 50 ml injection vial and seal. Accurately weigh and inject 15 µl 3-methyl-2-pentanone through the septum and reweigh to within 0.01 mg

L-1.6 Blank Solution

Weigh accurately 0.20 g of the blank into an injection vial. Add 5.0 ml of water and 1.0 ml of the internal standard solution. Heat at 60°C for 10 min and shake vigorously for 10 s.

L-1.7 Sample

Disperse 1 ml of a suitable antifoam emulsion, in 200 ml of water contained in a 1 000 ml 24/40 round bottom distilling flask. Add about 5 g of the sample, accurately weighed, and shake for 1 h on a wrist-action mechanical shaker. Connect the flask to a fractionating column and distil about 100 ml, adjusting the heat so that the foam does not enter the column. Quantitatively transfer the distillate to a 200 ml volumetric flask, fill to the mark with water and shake the flask to mix. Weigh accurately 8.0 g of this solution into an injection vial. Add 1.0 ml of the internal standard solution. Heat at 60°C for 10 min and shake vigorously for 10 s.

L-1.8 Calibration Solution

Weigh accurately 0.20 g of the blank into an injection vial. Add 5.0 ml of the water and 1.0 ml of the internal standard solution. Weigh the vial accurately to within 0.01 mg. Inject a known volume of the component of interest through the septum and again reweigh the vial. Heat at 60°C for 10 min and shake vigorously for 10 s.

L-2 PROCEDURE

Place the sample, blank and calibration samples in the sample tray of the head-space gas chromatograph - FID system. Analyze using the following analytical conditions.

Column: Fused silica, length 0.8 m, i.d. 0.53 mm, coated with DB-wax, film thickness 1 µm

Coupled with: Fused silica, length 30 m, i.d. 0.53 mm, coated with DB-1, film thickness 5 µm.

Conditions

Carrier gas: Helium

Flow rate: 208 kPa, 5 ml/min

Detector: FID

Temperatures

Injector: 140°C

Oven conditions: 35°C for 5 min, then 5°C/ min to 90°C, then 6 min at 90°C

Detector: 300°C

Head space sampler

Sample heating temperature: 60°C

Sample heating period: 10 min

Syringe temperature: 70°C

Transfer temperature: 80°C

Sample gas injection: 1.0 ml in split mode

Approximate retention time of methanol, ethanol and isopropanol are 2.93, 4.09 and 5.23 min respectively.

L-3 CALCULATION

$$\text{Component per injection vial in mg} = \frac{A \times B \times C}{50}$$

where

A = relative peak area of the component concerned,

B = mg internal standard C = calibration factor, and

C = calibration factor = $D \times 50 / [E \times (F - G)]$

where

D = mg component weighed,

E = mg internal standard,

F = relative peak area of component for the calibration solution, and

G = relative peak area of the same component for the blank solution.

Bureau of Indian Standards

BIS is a statutory institution established under the *Bureau of Indian Standards Act*, 1986 to promote harmonious development of the activities of standardization, marking and quality certification of goods and attending to connected matters in the country.

Copyright

BIS has the copyright of all its publications. No part of these publications may be reproduced in any form without the prior permission in writing of BIS. This does not preclude the free use, in the course of implementing the standard, of necessary details, such as symbols and sizes, type or grade designations. Enquiries relating to copyright be addressed to the Director (Publications), BIS.

Review of Indian Standards

Amendments are issued to standards as the need arises on the basis of comments. Standards are also reviewed periodically; a standard along with amendments is reaffirmed when such review indicates that no changes are needed; if the review indicates that changes are needed, it is taken up for revision. Users of Indian Standards should ascertain that they are in possession of the latest amendments or edition by referring to the latest issue of 'BIS Catalogue' and 'Standards : Monthly Additions'.

This Indian Standard has been developed from Doc No.: FAD 08 (1996).

Amendments Issued Since Publication

Amend No.	Date of Issue	Text Affected

BUREAU OF INDIAN STANDARDS

Headquarters:

Manak Bhavan, 9 Bahadur Shah Zafar Marg, New Delhi 110002
Telephones : 2323 0131, 2323 3375, 2323 9402 *Website*: www.bis.org.in

Regional Offices: *Telephones*

Central : Manak Bhavan, 9 Bahadur Shah Zafar Marg
NEW DELHI 110002 — 2323 7617, 2323 3841

Eastern : 1/14 C.I.T. Scheme VII M, V. I. P. Road, Kankurgachi
KOLKATA 700054 — 2337 8499, 2337 8561, 2337 8626, 2337 9120

Northern : SCO 335-336, Sector 34-A, CHANDIGARH 160022 — 260 3843, 260 9285

Southern : C.I.T. Campus, IV Cross Road, CHENNAI 600113 — 2254 1216, 2254 1442, 2254 2519, 2254 2315

Western : Manakalaya, E9 MIDC, Marol, Andheri (East)
MUMBAI 400093 — 2832 9295, 2832 7858, 2832 7891, 2832 7892

Branches: AHMEDABAD. BANGALORE. BHOPAL. BHUBANESHWAR. COIMBATORE. DEHRADUN. FARIDABAD. GHAZIABAD. GUWAHATI. HYDERABAD. JAIPUR. KANPUR. KOCHI. LUCKNOW. NAGPUR. PARWANOO. PATNA. PUNE. RAJKOT. VISAKHAPATNAM.

Published by BIS, New Delhi